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INTRACELLULAR ORIGIN OF HORMONE-SENSITIVE LIPOLYSIS IN THE RAT

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SUMMARY

The hormone-stimulated hydrolysis of endogenous triglycerides in heart and adipose tissue was found to be inhibited by chloroquine, which is known to accumulate in lysosomes and to inhibit the lysosomal degradation of protein and cholesterolesters.

When the triglyceride depôt in heart cells was increased by feeding rats a diet enriched in erucic acid for three days prior to in vitro perfusion of the heart, the spontaneous and the norepinephrine stimulated rates of lipolysis were both found to be increased. Both were inhibited by chloroquine. Analysis of trioleoylglycerol hydrolysis in heart homogenates after in vitro heparin perfusion revealed the virtual absence of neutral lipase in contrast to an acid lipase activity. The results of this study suggest that lipolytic activity of lysosomal origin is the main source of hormone-sensitive endogenous triacylglycerol hydrolysis.

Catecholamine activation of lipolysis in adipose tissue has been well documented[1,2]. The enzyme has been partially purified[3]. Adipose tissue also contains lipoprotein lipase, which may be removed by heparin treatment. In heart, catecholamine stimulated lipolysis is also known[4,5], as well as the presence of (heparin-releasable) lipoprotein lipase. In heart homogenates Björntorp and Furman[6] distinguished two lipases: a "tissue lipase" with a pH optimum of 6.8 and lipoprotein lipase with pH optimum of 8.5. In liver, two triacylglycerol hydrolases may also be distinguished: a lysosomal lipase, which has been purified[7], and a heparin-releasable enzyme. The heparin-releasable enzymes of extrahepatic and hepatic tissues are different[8,9,10], but it is not certain whether the "tissue lipases" are different. It has been shown by Teng and Kaplan[7] that the purified enzyme has a pH optimum of 4.0, but that highly stimulatory cardiolipin shifts the pH optimum to 4.5. Hence the lipid environment of the membrane-bound enzyme codetermines the pH optimum. All "tissue lipase systems" may be stimulated by cyclic AMP, as judged by the catecholamine stimulation of endogenous lipid hydrolysis, which was mentioned above. Guder et al.[11] reported glucagon stimulation of ketogenesis in liver and found also that the hormone increased the lability of acid lipase in in vitro perfused livers of starved rats. Therefore, it is possible that cyclic AMP stimulates the lysosomal action. Ashford and Porter[12]

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demonstrated a lysosomal enzyme in autophagosomes after perfusion of liver with glucagon, while Deter and De Duve[13] reported that glucagon not only increased the formation of autophagic vacuoles, but also the fragility and osmotic sensitivity of lysosomes.

A suitable tool to identify the involvement of lysosomes in degradative processes seems to be (pre)treatment of tissues with chloroquine, a weakly basic drug, which mainly accumulates in lysosomes and binds to the lysosomal membranes[14,15]. It has been used to demonstrate lysosomal protein degradation[16,17] and cholesterolester hydrolysis[18]. The present paper describes experiments with chloroquine on heart and adipocytes, it discusses determinations of lipolytic activities in heart and liver, from which the conclusion is drawn that "tissue lipases" of heart, adipose tissue and liver are all attached to intracellular (lysosomal?) membranes. In sonicated homogenates of heart the enzyme itself is not sensitive to chloroquine, while in the intact organ full sensitivity is obtained.

METHODS

For the heart perfusion experiments male Wistar rats were fed with normal rat pellets or put on a 3-day diet containing 40% of the total calories as rapeseed oil. After pentobarbital anaesthesia (70 mg/kg body weight), the hearts were quickly excised and perfused retrogradely, as described earlier[19]. The hearts were electrically paced at a rate of 300 beats/min. The mean coronary flow rate in the presence of norepinephrine was 9.0 ± 1.3 ml/min^{-1}. g wet wt^{-1}.

Glycerol and lactate, released from the heart of both groups of rats during control (15 min) perfusion and during continuous infusion of norepinephrine (final concentration 10^{-7} M) were determined fluorometrically[20]. Careful calibration of fluorescence was carried out in each experiment by adding a known amount of NADH. Chloroquine diphosphate was dissolved in saline and injected intraperitoneally (75 mg/kg body weight) two hours prior to perfusion of the hearts[21]. Preperfusions, with 50 µM chloroquine, lasted 30 min to ensure sufficient "wash-in" of the drug.

The release of lipoprotein lipase activity from the hearts was followed during perfusion with Tyrode-buffer containing 1% (W/v) fatty-acid-free bovine serum albumin, 5 I.U. heparin/ml and 5.10^{-7} M norepinephrine. Five successive 20 ml effluent fractions were collected in precooled glycerol[22] (final concentration 20% V/v) and lipase activity was estimated immediately.

Adipocytes were isolated from epididymal fat pads with collagenase according to Rodbell[23].

Lipolytic activities at pH 8.2 were tested with 100 µl 20% (W/v) glycerol containing perfusates or 10% (W/v) heart homogenates (a Polytron PT10 homogenizer was used at 4000 rev./min) in perfusion medium in a final volume of 250 µl by incubation with Intralipid (Vitrum, Sweden), 0.5% Intralipid (containing 0.5% W/v soybean oil and 0.03% egg phospholipid) was made radioactive by the manufacturer by the addition of glyceroltri[9,10(n)-^{3}H]oleate. 1.9 ml of this Intralipid was mixed with 0.5 ml 10% non-labeled, commercially available, Intralipid to give a triglyceride solution of about 28 mM (spec.act. 0.35 µCi/µmole). 40 µl of this substrate was used in each test. The final concentrations were: 40 mM Tris-HCl, 2.5% W/v bovine serum albumin, 0.8 mM $CaCl_2$, 0.15 M NaCl, 1.9 mM KCl, 8 mM $NaHCO_3$, 0.1 mM NaH_2PO_4, 0.4 mM $MgCl_2$, 4.4 mM glucose, 4.5 mM triglyceride, when indciated 8 µg pure apolipoprotein C_{II}/ml, and perfusate or heart homogenate.

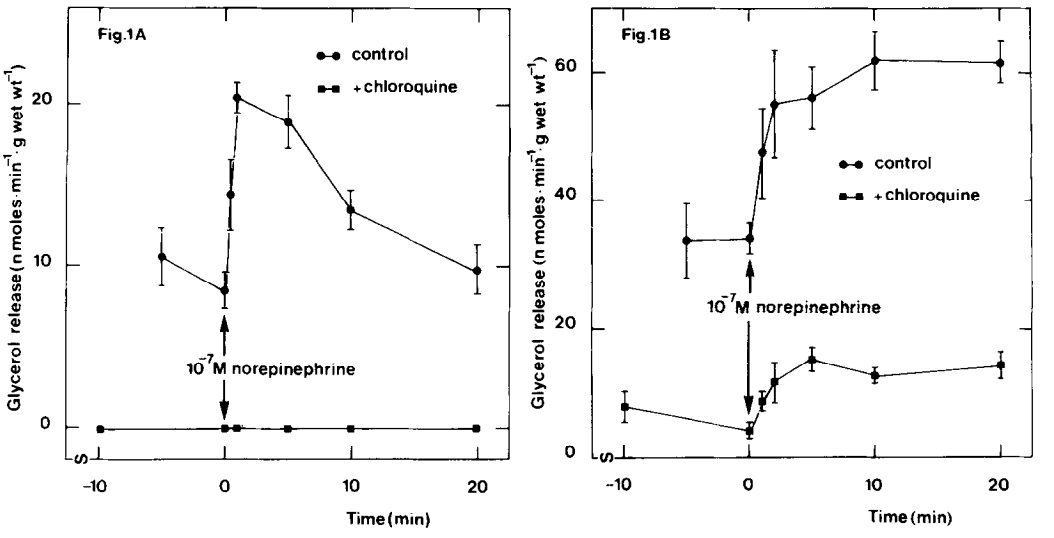

Fig. 1 A+B. Effect of 50 µM chloroquine on the glycerol release from isolated, perfused hearts of normal fed and rapeseed-oil fed rats during control perfusion and in the presence of 10^{-7} M norepinephrine. The results are the mean ± S.E.M. of four experiments.

Final pH 8.2. After 30 min at 37°C, the reactions were stopped with chloroform/methanol/heptane, followed by mixing with borate buffer of pH 10.5 to extract the oleate into the aqueous phase, exactly as described[24]. After centrifugation, 1 ml was counted in a liquid scintillation counter.

Lipolytic activity at a final pH of 5.2 was tested with 100 µl W/v heart homogenate (as above) in 250 µl final volume. Other additions (final concentrations) were 50 mM glyceroltri[1-^{14}C]oleate (spec.act. 0.03 µCi/µmole), 1.5% W/v gum acacia and 125 mM cacodylate buffer pH 5.2. These additions were sonicated (3 x 1 min at 21 kHz per ml emulsion). The enzyme preparation was also sonicated in this case (1 min/ml at 21 kHz). In 3 experiments the effect of 50 µM chloroquine was also tested as mentioned in the text. After incubation for 30 min at 37°C the reaction was stopped and counting performed as mentioned above.

Lipolytic activities were expressed as nmoles fatty-acid released per min (mU) per g wet wt. Results are given as mean ± S.E.M.

RESULTS

In vitro perfusions of rat hearts with chloroquine

From Fig. 1A it can be seen that in hearts from normal fed rats the glycerol production rate is about 10 nmoles/min/g wet wt., which is doubled immediately following the addition of 0.1 µM norepinephrine. Both the spontaneous and the hormone-stimulated lipolysis are completely inhibited by adding chloroquine (50 µM) to the perfusion medium. The effect of chloroquine was found to be fully reversible (not shown). The rate of lipolysis can be increased 3-fold

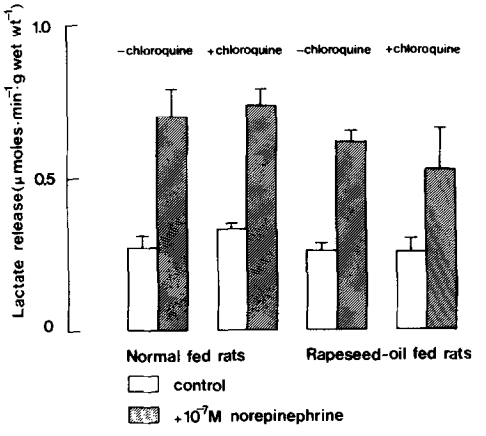

Fig. 2. Effect of chloroquine (50 μM) on basal and norepinephrine-stimulated lactate release from isolated, perfused hearts of normal fed and rapeseed-oil fed rats. Control values represent the lactate levels at the end of the pre-perfusion period. Norepinephrine-stimulated values represent the lactate levels 5 min after the infusion of the hormone. The results are the mean ± S.E.M. of four experiments.

(Fig. 1B) by increasing the triglyceride content of the heart by feeding the animals for 3 days with a diet rich in erucic acid[25] (40 cal%). Apparently the rate of lipolysis is affected by the lipid store of the organ. Again both the spontaneous and the norepinephrine-stimulated rates of lipolysis were strongly although not completely inhibited by chloroquine. Chloroquine does not interfere with glycogenolysis as judged by the absence of an effect on the rate of lactate production (Fig. 2). Therefore it may be concluded that the effect of chloroquine on lipolysis is specific.

Effect of chloroquine on lipolysis in adipocytes

Norepinephrine-stimulated lipolysis in adipocytes, is also highly sensitive to chloroquine inhibition (Table I). Doubling the amount of chloroquine or using 1 μM norepinephrine instead of 0.2 μM did not influence the results. Dibutyryl cyclic AMP-stimulated lipolysis was also inhibited by chloroquine (not shown).

Relative contributions of triacylglycerol hydrolases in heart and liver

It has been noted by a number of authors, including our group[26] that in vitro perfusion of rat heart with heparin only partially removes lipoprotein lipase. If a proper coronary flow rate is maintained (by millipore filtration of the albumin and heparin containing perfusion medium prior to use; electrical stimulation of

TABLE I

EFFECT OF CHLOROQUINE ON LIPOLYSIS IN ADIPOCYTES[a]

Additions	n[b]	% of norepinephrine-stimulated activity
none	5	3.0 ± 1.1
25 μM chloroquine	5	3.1 ± 1.6
0.2 μM norepinephrine	5	100
0.2 μM norepinephrine +25 μM chloroquine	5	32.9 ± 9.7

[a] The adipocytes were incubated at 37°C in Krebs-Ringer bicarbonate buffer, equilibrated with a gas mixture of 95% O_2 and 5% CO_2 (v/v) to give a final pH of 7.4. The reactions were stopped after 15 min by cooling, followed by centrifugation. In the infranatant glycerol was determined as described under Methods.

[b] n = number of experiments.

the heart and a sufficiently high perfusion pressure (100 cm H_2O)) 89% of the lipoprotein lipase is removed in 11 min of perfusion with 5 I.U. heparin/ml (Table II). The residual trioleoylglycerol hydrolase activity in the heart homogenate, measured at pH 8.2, can be characterized as lipoprotein lipase, since the activity may be stimulated by apolipoprotein C_{II}, while the non-stimulated activity is inhibited by the lipoprotein lipase inhibitor protamine sulfate[9]. We tested for a hormone-sensitive lipase in the homogenate by including 0.5 μM norepinephrine in the perfusion medium, but no significant trioleoylglycerol hydrolase activity can be found at pH 8.2 except lipoprotein lipase. The trioleoylglycerol hydrolase activity at pH 5.2 (broad pH optimum between 5 and 6.5 - data not shown) is much higher. This (acid) lipase may be of lysosomal origin since a lysosomal lipase does exist in heart, as found by others and as judged by the chloroquine inhibition observed in Fig. 1. The lipase activity at pH 5.2, as measured in sonicated homogenates, was not sensitive to 50 μM chloroquine (Table II; comparison of paired experiments revealed no inhibition).

DISCUSSION

In liver most of the neutral (or alkaline) trioleoylglycerol hydrolase activity is removed by heparin perfusion[27], provided the liver is perfused at a sufficiently high flow rate. The small residual activity (measured at pH 8.5) is completely due to heparin-releasable lipase, since the residual activity is

TABLE II

DIFFERENTIATION OF MYOCARDIAL LIPOLYTIC ACTIVITY

	apo C_{II}	n[c]	mU/g wet wt
heparin-releasable LPL[a] in perfusate	+	9	483 ± 132
non-releasable LPL in heart	+	9	60.4 ± 4.0
non-releasable LPL in heart	-	9	18.4 ± 2.1
non-releasable LPL in heart + 50 µg/ml protamine sulfate	-	9	9.0 ± 0.9
acid lipase[b]	-	9	103.4 ± 5.8
acid lipase + 50 µM chloroquine	-	3	91.0 ± 3.6

[a] Lipoprotein lipase (LPL) is defined as apolipoprotein C_{II} (apo C_{II}) dependent trioleoylglycerol hydrolase activity and measured at pH 8.2, as described under Methods.

[b] Acid lipase was measured in the absence of apo C_{II} with trioleoylglycerol at pH 5.2, as described under Methods.

[c] n = number of experiments.

inhibited by an antibody against the heparin-releasable enzyme. Therefore there is no other neutral (alkaline) triacylglycerol hydrolase. There is, however, an acid lipase which has been highly purified[7]. In heart, a similar situation exists: besides heparin-releasable lipase there is only an (acid) tissue lipase (Table II). This agrees with the observations of Björntorp and Furman[6], who found two lipases, and observed a similarity between the tissue lipase and the adipose tissue lipase. The similarities may now be extended. The tissue lipase activities are hormone-sensitive and they are inhibited by chloroquine, a known inhibitor of lysosomal activity. Therefore we ask whether the hormone-sensitive lipase in all tissues is embedded in the membrane of lysosomes and/or autophagic vacuoles. This question may help to resolve the mechanism of hormone-sensitivity. Whether activation is due to cyclic-AMP-dependent membrane phosphorylation or enzyme phosphorylation cannot yet be concluded[3]. The highly stimulatory effect of negatively charged phospholipids on the purified lysosomal lipase from liver[7], raises the possibility that membrane phosphorylation can influence the regulatory membrane environment of the enzyme, which may also be influenced by the weakly basic inhibitor chloroquine.

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